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(54) Title: BIO-CHIP, PHOTOLUMINESCENT METHODS FOR IDENTIFYING BIOLOGICAL MATERIAL, AND APPARATUSES FOR USE WITH SUCH METHODS AND BIO-CHIPS

(57) Abstract: A method detects binding of molecules, advantageously without tagging molecules in the sample. A sensor is used in which is included a single stranded nucleic acid sequence and a photoluminescent material in respective layers. After the sensor is exposed to a biological sample for sufficient time for its single stranded nucleic acid sequence to bind to a material of interest, photoluminescence from the sensor can be measured. An apparatus for tagging-free detection of binding of molecules also is provided. Methods of making tagging-free sensors are provided. Also, tagging-free methods to detect binding of antigens and related devices are disclosed.

BIO-CHIP, PHOTOLUMINESCENT METHODS FOR IDENTIFYING BIOLOGICAL MATERIAL, AND APPARATUSES FOR USE WITH SUCH METHODS AND BIO-CHIPS

DESCRIPTION

Field of the Invention

The invention generally relates to nucleic acid, and more particularly, to binding of single stranded nucleic acid with biological material of interest in a sample to identify the material.

BACKGROUND OF THE INVENTION

Nucleic acids such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein nucleic acid (PNA) are fundamental components of matter of living organisms. Nucleic acids generally speaking consist of certain constituent parts, or base pairs. The permutations in which these base pairs may be arranged is vast. Nucleic acid sequence analysis, i.e., determining the identity and sequence of the base pairs of a nucleic acid sample, is an important technology. Deciphering nucleic acid sequences is important for disease diagnosis, drug design and understanding of various biological mechanisms. Before 1996, traditional methods laboriously "read" the gene sequencing one base pair at a time.

Around 1996, Affymetrix developed a massively parallel sequencing approach, using a DNA chip with which several base pairs can be read simultaneously. A monolayer of specific single stranded DNA (ssDNA) fragments is assembled on an array of pixels (~1-100 μm^2). The type of

relies on DNA sequencing and other protein sequencing would be helped by expedited rapidity of sequencing, simplified sequencing, and/or enhanced precision and accuracy. Also, a small portable device useable in a doctor's office to check, for example, if a patient may eventually develop cancer or how fast the body is likely to break down a specific anti-cancer drug have been generally theorized as of interest. For all these applications, a tool that can perform a nucleic acid analysis of a small size sample for several specific genes (at low concentrations) is highly desirable, but not conventionally provided. Rather, the conventional bio-chip methods undesirably require tagging of the sample plus other disadvantages (such as expensive manufacturing methods, uncertainty in the number of fragments per pixel, etc.).

SUMMARY OF THE INVENTION

It therefore is an object of this invention to provide methods and products for detecting the hybridization state of a nucleic acid molecule, without needing to tag the sample. The invention can be used to perform sequence analysis of unknown nucleic acids, such as DNA sequences. Several genes can be probed simultaneously. Sequencing according to the invention is relatively simple and quick, while providing precise and accurate sequencing information. The invention provides a bio-chip and other products for simultaneously analyzing one or more specific nucleic acid fragments (such as genes) in a solution.

In order to accomplish these and other objects of the invention, the present invention in a preferred embodiment provides a tagging-free method to detect binding of molecules, comprising the steps of: (A) providing a sensor comprised of a first layer including a single stranded nucleic acid sequence and a second layer including a photoluminescent material; (B) exposing said sensor to a biological sample for sufficient time for said single

In another preferred embodiment, the invention provides a method of making a tagging-free sensor, comprising: contacting a single stranded nucleic acid sequence with a photoluminescent material. A particularly preferred embodiment of such an inventive method includes depositing photoluminescent material on a substrate to form a surface, and thereafter modifying the surface by ion exchange treatment with a metal salt, followed by ion-embedding, followed by exposing the ion-embedded material to reactive media to form photoluminescent particles.

In the above-mentioned methods, products and apparatuses, a particularly preferred embodiment of the invention uses DNA, RNA and/or PNA as the single stranded nucleic acid. In an especially preferred embodiment, the first layer comprises an ssDNA monolayer. In a particularly preferred embodiment, the sensor comprises ssDNA as said first layer grafted onto the second layer.

In a preferred embodiment, the nucleic acid sequence is between 5 and 200 base pairs. In an especially preferred embodiment, the sequence is about 25 base pairs. Another particularly preferred embodiment provides a discontinuous first layer comprising different nucleic acid sequences in different sections of said first layer.

Further, another preferred embodiment of the invention provides a tagging-free method to detect binding of antigens, comprising the steps of: (A) providing a sensor comprised of a first layer including an antibody and a second layer including a photoluminescent material; (B) exposing said sensor to a biological sample for sufficient time for said antibody to bind to an antigen of interest in said biological sample; (C) measuring photoluminescence from said sensor. In a particularly preferred embodiment, the first layer is discontinuous and comprises different antibodies.

In another preferred embodiment, the invention provides an apparatus for tagging-free detection of antibody binding, comprising: a light

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, aspects and advantages will be better understood from the following detailed description of the preferred embodiments of the invention with reference to the drawings, in which:

Figure 1 is a flowchart depicting a method according to the present invention.

Figure 2(a) is a cross-sectional view of a device according to the invention. Figures 2(b) and 2(c) are enlarged views of part of Figure 2(a), with Figure 2(b) showing unbound single stranded nucleic acid material according to the invention and Figure 2(c) showing bound nucleic acid material according to the invention.

Figures 3(a), (b) and (c) together are a series of cross-sectional views showing probing according to the invention.

Figures 4(a), (b), (c) and (d) depict device fabrication with in-situ nanocomposite formation according to the invention.

Figure 5 shows an Atomic Force Micrograph of a nano-composite structure according to the invention.

Figures 6(a) and 6(b) show an optical micrograph of a selectively modified polystyrene surface according to the invention.

Figure 7 shows fluorescent spectra from polystyrene used in a photoluminescent-polymer containing embodiment of the invention.

Figure 8 shows a monolayer of polystyrene/polybutadiene block copolymer spheres on an exemplary silicon surface according to the invention.

Figure 9 is a graph of etching behavior of polybutadiene as a function of exposure time for an exemplary block copolymer according to the present invention.

Figure 10 is a graph of the effect of surface treatment on contact angle of water, for exemplary materials according to the present invention.

can be detected without using conventional tagging. The invention uses a material that is photoluminescent, which broadly includes a material that glows at any wavelength or that luminesces because of photons. The invention uses application of light, and the light input may be ultraviolet, visible, infrared or other light, without limitation.

In an exemplary embodiment of such an inventive tagging-free method, a single stranded nucleic acid material of known sequence is provided. Examples of the single stranded nucleic acid material are DNA, RNA and PNA. The single stranded nucleic acid sequence may be of any length, with a preferred example being about 25 bp; however, the single stranded nucleic acid could range from 5 to 200 bp.

As seen with reference to Figure 1 of an exemplary detection system according to the invention, a measurement or observation **100** optionally may be made for a known single stranded nucleic acid, alone. A numerical measurement is not necessarily required, and a baseline measurement in the nature of a calibrating measurement or observation is within the present invention. The present invention is based on differential (rather than absolute) measurements or observations. In some embodiments, measurement or observation **100** may be eliminated.

After measurement/observation **100**, the known single stranded nucleic acid sequence is then exposed to a biological sample, such as a sample containing biological material for which information is desired as to whether material complementary to the known single stranded nucleic acid sequence is contained therein. For example, the sample may comprise denatured DNA, RNA or PNA of unknown identity.

For contacting the sample with the single stranded nucleic acid, known methods may be used, such as providing the single stranded nucleic acid on a bio-chip and pipetting a small amount of the sample solution into a well in the bio-chip. The conditions of contacting the biological sample with

detectible luminescence will result because of changes in the physical characteristics of the nucleic acid between its single stranded and bound state.

In a preferred embodiment of the invention, a known single stranded nucleic acid sequence is provided on a substrate, probe, bio-chip or other solid product, and the biological sample is contacted in liquid form with the solid product. In other embodiments of the invention, the unknown or to-be-sequenced biological material may be provided on a substrate, probe, bio-chip or other solid product and contacted with a liquid probe comprising sensor particles each including a known single stranded nucleic acid sequence attached to a photoluminescent material.

The inventive methods and products may be used to sequence nucleic acid molecules (such as DNA, RNA or PNA) or other proteins. For example, one or more specific DNA fragments (i.e., a gene) of unknown identity and sequence may be analyzed. The source materials for a biological sample to test are not particularly limited and may be any nucleic acid or protein-containing biological materials (such as blood, tissue, fingernail clippings, etc.). Raw biological material, such as blood, generally may be processed into testable fragments and put into solution by methods known to those skilled in the art.

The single stranded nucleic acid sequence used in the present invention is not particularly limited in the form in which it is used, and a form such as a monolayer may be used. A monolayer is particularly preferred.

In a preferred embodiment, the single stranded nucleic acid of the present invention is attached to a photoluminescent material. A preferred example of the attachment is by grafting. "Grafting" may include covalently bonding a portion of the single stranded nucleic acid to the photoluminescent material (e.g., by an amine linkage from lysine or histidine), or it may include physical adsorption on the photoluminescent material, or other joining mechanism that associates the single stranded nucleic acid and the

(reflectivity) changes. The amount of light reaching the polystyrene attached to the DNA may change by as high as 200%, for the following reasons.

DNA absorbs generally in the 260-265 nm range (ultraviolet), and shining such radiation on an (unbound) single stranded nucleic acid top layer results in absorption, reflection and scattering of the radiation. The left-over transmitted radiation travels into the photoluminescent material (such as polystyrene) causing it to give out radiation at a different wave length. For example, the photoluminescence in polystyrene is at about 325 nm. However, if the single stranded nucleic acid is bound so that it becomes double stranded, the nucleic acid now absorbs, scatters and reflects a different amount of excitation wavelength, so that the amount of radiation experienced by a photoluminescent underlying layer differs from the case before binding occurred. Correspondingly the photoluminescent material gives out different photoluminescence intensity. The refractive index changes, because of which, the reflection (reflectivity) changes. The amount of light reaching the photoluminescent material underneath may change by 200% or more. This is primarily due to the refractive index changes, because of which, the reflection (reflectivity) changes, resulting in significant change with respect to the excitation light into the photoluminescent layer. Thus, studying the amount of light from the photoluminescent material attached to the nucleic acid sequence provides a dramatically-different result for unbound versus bound nucleic acid sequences.

Part of a bio-chip device that is an exemplary form of the invention is shown in Figure 2(a). The device of Figure 2(a), in which optically active layer **2** comprises a photoluminescent material such as a nanocomposite or a photoluminescent polymer, may be used as follows The nanocomposite which is a photoluminescent material may be provided in a matrix material, with the matrix material optionally being photoluminescent at the excitation wavelength. Figure 2(b) shows an enlarged view of part of

techniques known to those skilled in the art, and used in other testing or applications.

Probing according to the invention is a measurement of change in photoluminescent light, as generally shown in Figure 3, which relates to absorption for an inventive method according to Figure 1 or inventive device according to Figure 2(a). With reference to Figure 3, I_0 is the intensity of incident UV light at wavelength 265 nm (which is nominally the absorption maxima of DNA). As the UV radiation is absorbed by the layer of photoluminescent material **2**, the photoluminescent particles emit visible light of intensity I_1, I_2 or I_3 (which represent output intensity with no DNA graft (i.e., DNA immobilized on the surface by at least one covalent bond). If $\Delta I = I_2 - I_3$ can be measured, the occurrence of any hybridization reaction can be determined. The measurement of ΔI can be performed in-situ in the solution because the attenuation of I_0 due to absorption by the solution is constant and absorption by DNA and solution in the visible range is negligible.

Probing is depicted in Figures 3(a), (b) and (c), for the device shown in Figure 2(a), in a fiber optics mode. I_1 in Figure 3(a) is the photoluminescent intensity from optically active layer **2** which in this example is a nanocomposite for incident UV radiation, I_0. The output intensity is reduced to I_2 on deposition of ssDNA **3** (Figure 3(b)) that further reduces UV radiation intensity incident on the optically active layer **2** which is a nanocomposite. The intensity is further reduced when the ssDNA **3** is hybridized to form dsDNA **33** (Figure 3(c)). The resultant photoluminescent intensity for the device according to Figure 3(c) is I_3. The relevant signal is $\Delta I = I_2 - I_3$.

The device of Figure 2(a) optionally contains a reflective layer such as gold layer **4** to enhance reflection, depending on the end use. An optional reflective layer is preferably composed of a non-oxidizing material, of which gold is a preferred example. For example, as shown in Figure 2(a),

sensors according to the invention have applications as on-line sensors for PCR.

Devices comprising known single stranded nucleic acid sequences for use in practicing the invention may be easily fabricated, such as by the method shown in Figures 4(a) through (d). Examples of a device according to the present invention are a probe, a bio-chip, and other products useable in tagging-free sequencing.

For use in the present invention, known single stranded nucleic acid sequences of length may be used, with sequences of length from about 5 bp to about 200 bp being preferred, and a sequence of about 25 bp being most preferred.

To make an exemplary device according to the present invention, a single stranded nucleic acid sequence (preferably of known sequence) may be attached to a photoluminescent material. Attachment techniques for nucleic acids are known to those skilled in the art, including, without limitation, grafting, immobilization, electrovalent attachment, covalent attachment, adsorption, van der Waals attachment, etc. A preferred attachment mechanism is to covalently attach the nucleic acid sequence to the photoluminescent material. The attachment may be to directly attach the nucleic acid to the photoluminescent material or may be to indirectly attach the nucleic acid to the photoluminescent material, such as by interposing a linker or adhesion promoter.

The single stranded nucleic acid in a preferred example according to the invention is provided directly or indirectly on a substrate **1** (Figure 2(a)), the substrate not being particularly limited. A reflecting or nonreflecting substrate may be used, with a nonreflecting substrate being preferred. The substrate **1** is not particularly limited and may be selected based on the end-use probe method. A substrate is not required to be used in the present invention.

such that no nucleic acid adsorption other than the intended nucleic acid attachment occurs.

However, the surface may be selectively modified to allow for controlled nucleic acid grafting where the density of molecules per unit area can be regulated. The attachment density preferably is low enough to avoid entanglement of adjacent chains and to allow for enough chain mobility for fast hybridization kinetics in solution. Preferably the density should be high enough for large change in complex refractive index of the nucleic acid layer as it changes from single stranded to double stranded. Polystyrene is an example meeting these criteria.

Preferred production methods for making devices according to the present invention include nanocomposite production procedures, self-alignment production procedures, nano-particle nano-array fabrication, and polymer blob/nanoparticle fabrication.

An example of a nanocomposite production method is as follows. As shown in Figure 4(a), a nanocomposite film is deposited on a rigid substrate (such as quartz or glass). To deposit a smooth nanocomposite film shown in Figure 4(b), an in-situ three-step procedure is used, in which surface modification is followed by ion exchange with an appropriate metal salt. Next, the ions are subsequently embedded and then exposed to reactive media to form photoluminescent particles. During the particle-embedding, the polymer surface is regenerated. The resultant structure is shown in Figure 4(b), with Figure 5 showing an Atomic Force Micrograph (AFM) of a nano-composite structure corresponding to Figure 4(b) and according to the invention. The surface studied is 1 by 1 μm in tapping mode where both the topography (i.e. contrast is height) and damping (i.e., contrast is local hardness) are mapped. Comparing the three types of samples clearly indicates that the particle are formed and the embedded particles are smaller than when the sulfonation takes place without any diffusion of ions in the

Figure 4 thus shows in-situ nanocomposite formation according to the invention, starting with a highly smooth polystyrene film deposited on a substrate. The film is then processed in three steps to produce photoluminescent particles, with ZnS:Mn particles produced. The surface is regenerated to produce polystyrene at the surface. The film is exposed to similar surface modification to functionalize the surface with acidic or basic moieties to attach to the 5' or 3' end, respectively.

As shown in Figure 2(a), onto a substrate **1** optionally there is provided a gold electrode **4**. After forming the electrode **4**, a nancomposite comprising a polymer and photoluminescent particles is formed on the substrate **1** and electrode **4**. A solution of polymer and photoluminescent particles may be used. As examples of the photoluminescent particles may be mentioned particles that when exposed to UV light (about 265 nm wavelength), emit visible light (e.g., green), such as ZnS doped with Mn (i.e., ZnS:Mn).

In an embodiment in which a nanocomposite is used as the optically active layer **2**, nanocomposite is formed on the substrate. With reference to Figure 1, ssDNA **3** is grafted onto the nanocomposite. Preferably, the nanocomposite surface has been modified to allow for selective grafting thereon of ssDNA. The selective modification makes possible fabricating a chip with large combinatorial of ssDNA that can be used with simple micropipette dispensing.

After any optional pre-grafting surface modification, the ssDNA **3** layer is grafted, such as by an appropriate coupling linkage, such as difunctionalized organic compounds where one end will react with a (surface modified) polystyrene surface and the other end will attach to the 3' or 5' end of the ssDNA. Examples of such organic compounds are commercially available from Sigma Chemical Co. (St. Louis, Missouri) and Molecular Probes, Inc. (Eugene, Oregon).

vicinity of 265 nm wavelength. For example, the film may comprise polystyrene. The film may be deposited on an appropriate substrate depending on the intended end-use of the probe application. When the device is exposed to UV light (~265 nm wavelength), the photo-active polymer film will emit light and the device may be used as set forth above for a device in which the optically active layer **2** is a nanocomposite.

Figure 7 shows typical fluorescent spectra from polystyrene under various conditions, when used in a device according to the present invention. The dark curve with lower intensity is the luminescence spectra of polystyrene coated with a monolayer of ssDNA. The signal is intensified as the ssDNA is converted to dsDNA. The peak intensity increases and the peak position is red-shifted. The difference in the intensity of the fluorescent light before and after the conversion of ssDNA to dsDNA is defined as the contrast. The typical contrast shown in Figure 7 is significant to decipher the ssDNA to dsDNA transformation. This change in intensity (i.e., contrast) of the emitted light may be recorded by several methods, such as (i) a confocal microscope via a CCD camera; and/or (ii) a fiber-optic method. A fiber-optic integrated optics approach will allow for on-line measurement during the hybridization process in solution.

Fabrication of a photoluminescent polymer-containing device may be as follows. A surface modification method may be used, to allow for selective grafting of ssDNA on the polymer surface. Particularly, the selective modification allows fabricating a chip with large combinatorial of ssDNA using simple micropipette dispensing. With reference to Figure 2(a), the structure is composed of two active layers: the top layer of grafted ssDNA over an optically active material layer which in this embodiment is a fluorescent polymer layer. The nature of the substrate and other structure depends on the probe method. Two typical, but not limiting, probe methods are: (i) in the reflection mode, there is a third layer of reflective Au to deflect

in solution. Also, the density preferably is high enough to result in a large absorption by the DNA layer. One exemplary polymer that fits these criteria is polystyrene. Figures 6(a) and 6(b) show selective modification of polystyrene, with only the area exposed being wetting and suitable for single stranded nucleic acid strands to graft thereto.

Thus, a DNA chip according to the invention may be fabricated using self-assembly techniques, which are less expensive than lithography used in traditional methods of making DNA chips. Self-assembly is significantly less expensive than traditional lithography-based DNA chip fabrication methods. The self-alignment property of ssDNA is exploited, particularly, the functional polar region being surrounded by non-polar area. A reliable and relatively fast manufacturing process may be accomplished, with many depositions per minute possible.

Another alternative for making devices according to the present invention is to decorate nanoparticles on a block copolymer template. A block copolymer comprising at least two chain types is deposited on a substrate. The deposition is performed either by a solution process or a solid-forming process. The block-copolymer film is thermally processed to separate the nanophase into discrete phases of the minority polymer. The structure of the discrete phase may be nano-spheres or cylinders with nano-scale diameter or other (more) complex geometries depending on constituents of the block copolymer. The important property of the film is a structure with phase-separated regions with characteristic dimensions in the 5-500 nm scales.

Next, the block copolymer is subjected to a surface treatment that etches the surface to expose the discrete phase and in the same process activates the surface of the discrete phase. However, the etched "matrix" polymer is not activated. Subsequently, the structure is exposed to precursor solution of inorganic and/or organo-metallic salts in a solvent system that

lattice. As an example, minority phase polymer segment **8A** may be polystyrene (PS) or poly(methyl methacrylate) (PMMA) and majority phase **8B** may be polyisoprene or polybutadiene.

In Figure 15(b), the block copolymer thin film **8** is spin cast and annealed to form a monolayer of the microphase separated spheres. Then the matrix is etched by ozone or plasma to form isolated A-spheres **8a**. Subsequently the sphere **8a** is annealed above the glass transition temperature of the polymer to form polymer "blob" **88** (see Figure 15(c)). In the next step, the surface **1a** of the substrate **1** is modified by anionic groups (see Figure 15(d)) to provide modified blobs **88a**. The group used for such modification may be a strong anion that forms a salt when exposed to an aqueous solution of Zn, Cd, Pb or Hg salts. Upon annealing (see Figure 15(e)) to form annealed blobs **88b** and exposure to H_2S, II-VI calcogenid nanoparticles **9** will be formed (see Figure 15(f)) in the blobs **88c**. To provide the highest photoluminescence , a typical nanoparticle may be doped, such as, ZnS:Mn or ZnS:Ag. The former has highest photoluminescence intensity around 265 nm which is close to the maximum absorption of nucleic acids in the UV-region.

In the next step, the blob surface is modified again by wet chemistry, plasma or corona to form moieties that can react with the 3' or 5' end of the nucleic acid chain. For example, if a hydroxyl group is formed by water plasma, the phosphate end will react to form a covalent bond. Thus the nucleic acid chain will be grafted on the polymer blob surface. Depending on the size of the nucleic acid, one or more chains can be grafted per blob. For a long chain over 50 bases, only one chain may be grafted per blob due to the lateral size of the chain (see Figure 16(a) which is an enlargement of Figure 15(g)). Figures 15(g) and Figure 16(a) show a final structure with a nanoparticle **9** in a blob **88d** and the single stranded nucleic acid **3a** graft. When such a surface is exposed to a mixture of single stranded nucleic acid

The production methods discussed above are by way of example, and are not limiting. For example, where ssDNA is mentioned, the methods may be applied for other nucleic acids.

Using any of the above-mentioned production methods, or any other suitable method, a bio-chip such as that of Figure 13 with an array of pixels **5** may be made. In a preferred embodiment, the invention provides a DNA chip for photoluminescent measurements at 10^4 pixels (sites). The same pixel may be repeated 100 times, at different locations, so that repeated measurements may be made, to establish statistical significance. For example, a 10^4 μm^2 size probe with structure shown, for example, as in Figure 16(a) may be fabricated. Each substrate may have a different single stranded nucleic acid probe. The UV light input and visible light out may be coupled in to the substrate by a typical integrated optical method, known to one skilled in the art. For example, optical fibers may be used. To improve the signal to noise ratio the incident beam may be modulated and the output signal may be locked-in at the same frequency. Such an integrated approach advantageously builds an on-line sensor where each fiber or fiber bundle has a type of single stranded nucleic acid different from the other fiber bundle. In this way, several single stranded nucleic acids may be probed in a mixture simultaneously, such as in a multi-gene probe.

A "digital" approach may be used in fabricating a bio-chip according to the invention, in which the probe sites are arranged on a periodic array with 10-50 nm scale periodicity. A discrete, periodic arrangement will allow for absolute nucleic acid count with high sensitivity and accuracy, and low noise/error that otherwise arises due to overlap of signals from adjacent fragments in an "analog" method. Compared to conventional bio-chip technologies, the inventive methods and products are useable with sample sizes 10^3-10^4 smaller. Also, the array fabrication may be based on a relatively

the PS spheres are bright compared to the PB matrix. The brightness of PB is attributed to presence of high atomic number Zn compared to low atomic weight materials (i.e., C and H) in the matrix. In Figure 11, ZnS nanoparticles on a block copolymer are shown, and notably the PS discrete islands are spherical and bright compared to the matrix indicating that the ZnS is confined only to PS. The random arrangement of PS spheres is due to over-etching of PB. Photo-luminescence (PL) spectra of the resultant sample shown in Figure 12, which shows photoluminescence from ZnS particles, indicate that the typical emission peak at ~420 nm is strongly blue shifted by over 100 nm indicating the presence of nanoparticles of ZnS. The strong blue shift is an indication of quantum confinement effects indicating a nominal size of <3 nm ZnS particles.

EXAMPLE 2

Using self-assembled block copolymer monolayers and high anisotropic etching, a structure is made by grafting ssDNA fragments (x) on a nanoparticle coated polymer blob. The blobs (made of polystyrene (PS)) are arranged periodically on a (silicon) substrate. The periodicity and size of the blobs is in the 10-20 nm range (see Figure 14(a)). The direct band gap semiconductor nanoparticles are embedded in-situ in the polymer to ensure nominally monodispersed size distribution and to simplify the process. The luminescence excitation will be tailored to the maximum at ~260 nm (close to the absorption maxima of DNA). As the ssDNA is grafted, the luminescent light in the visible range will be attenuated from I_0 to I_i. Thus by measuring the difference $\Delta I=I_f-I_i$ as the probe sites with x grafts are exposed to a ssDNA mixture the amount of y fragments can be quantitatively calculated. The calibration constant relating ΔI and number of y attached to the substrate is determined exactly since the number of x sites are known. To achieve measurable contrast, i.e., $\Delta I \sim 10\%$ change, the minimum ssDNA size is a 50 base pair sequence. For 50 base sequence ssDNA on 20 nm pitch array, only

CLAIMS

We claim:

1. A tagging-free method to detect binding of molecules, comprising the steps of:

(A) providing a sensor comprised of a first layer including a single stranded nucleic acid sequence and a second layer including a photoluminescent material;

(B) exposing said sensor to a biological sample for sufficient time for said single stranded nucleic acid sequence to bind to a material of interest in said biological sample;

(C) exposing said sensor to light and measuring photoluminescence from said sensor.

2. The tagging-free method of claim 1, wherein the single stranded nucleic acid is selected from the group consisting of DNA, RNA and PNA.

3. The tagging-free method of Claim 1 wherein said second layer is selected from the group consisting of aromatic polymers, doped or undoped metal oxides, sulfides, selenides, arsenides, tellurides, and nitride and phosphide nanocomposites.

4. The tagging-free method of claim 1 wherein said second layer comprises a matrix material, with said photoluminescent material associated with said matrix material.

5. The method of claim 4 wherein said photoluminescent material is embedded in said matrix material.

15. The tagging-free method of claim 1, wherein the second layer comprises a polymer.

16. The tagging-free method of claim 1, wherein the nucleic acid sequence is between 5 and 200 base pairs.

17. The tagging-free method of claim 16, wherein the sequence is about 25 base pairs.

18. The tagging-free method of claim 1, wherein the second layer has fluorescence when excited by light with a wavelength in the 200-700 nm range.

19. The tagging-free method of claim 1, wherein the sensor comprises ssDNA as said first layer grafted onto the second layer.

20. The tagging-free method of claim1, including providing a discontinuous first layer comprising different nucleic acid sequences in different sections of said first layer.

21. The method of claim 1, wherein said first layer is positioned on a first side of said second layer, and said measuring step measures photoluminescence from a second side of said second layer.

22. The method of claim 21, wherein said second side is opposite said first side on said second layer.

31. A method of making a tagging-free sensor, comprising:

contacting a single stranded nucleic acid sequence with a photoluminescent material.

32. The method of claim 31, including depositing photoluminescent material on a substrate to form a surface, and thereafter modifying the surface by ion exchange treatment with a metal salt, followed by ion-embedding, followed by exposing the ion-embedded material to reactive media to form photoluminescent particles.

33. A tagging-free sensor comprising a first layer including a single stranded nucleic acid sequence and a second layer including a photoluminescent material.

34. The sensor of claim 33, wherein the single stranded nucleic acid is selected from the group consisting of DNA, RNA and PNA.

35. The sensor of claim 33, wherein said second layer is selected from the group consisting of aromatic polymers, metal oxides and sulfides, and nanocomposites.

36. The sensor of claim 33, wherein the second layer comprises polystyrene.

37. The sensor of claim 33, wherein the second layer comprises zinc sulfide.

38. The sensor of claim 33, wherein the second layer comprises a nanocomposite.

47. An apparatus for tagging-free detection of antibody binding, comprising:

a light source;

a sensor having a first layer including an antibody and a second photoluminescent layer; and

a light detector.

48. A tagging-free sensor comprising a first layer including an antibody and a second layer including a photoluminescent material.

49. The sensor of claim 48, wherein the first layer is discontinuous and comprises different antibodies.

50. The sensor of claim 49, including different known antibodies.

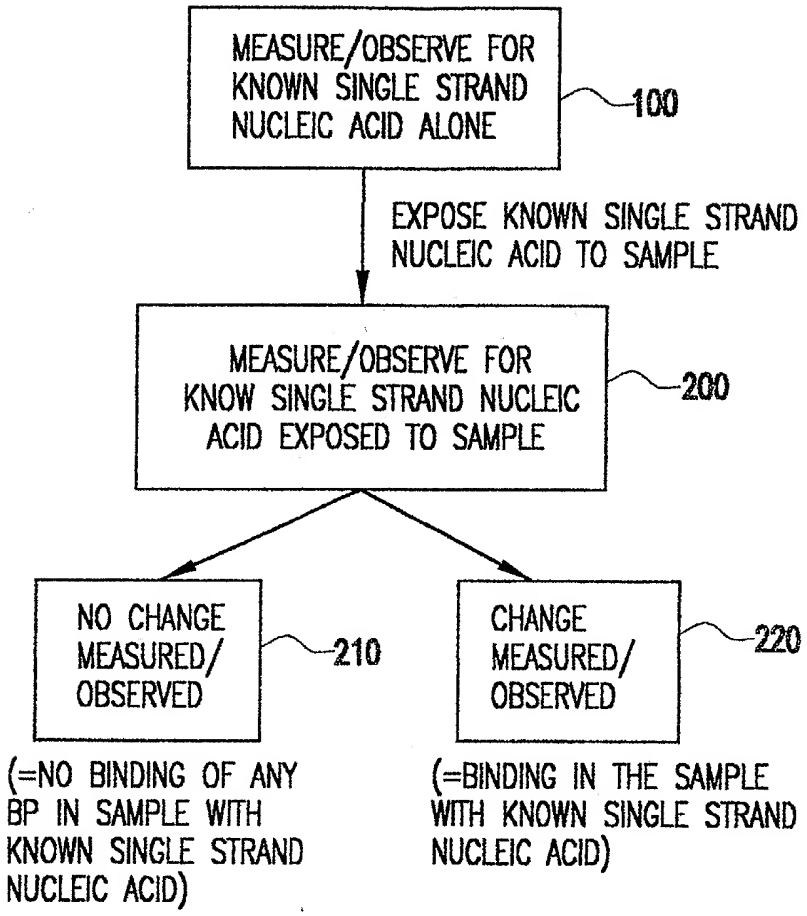

MEASURE/OBSERVE FOR KNOWN SINGLE STRAND NUCLEIC ACID ALONE
100
EXPOSE KNOWN SINGLE STRAND NUCLEIC ACID TO SAMPLE
MEASURE/OBSERVE FOR KNOW SINGLE STRAND NUCLEIC ACID EXPOSED TO SAMPLE
200
NO CHANGE MEASURED/ OBSERVED
210
(=NO BINDING OF ANY BP IN SAMPLE WITH KNOWN SINGLE STRAND NUCLEIC ACID)
CHANGE MEASURED/ OBSERVED
220
(=BINDING IN THE SAMPLE WITH KNOWN SINGLE STRAND NUCLEIC ACID)

FIG.1

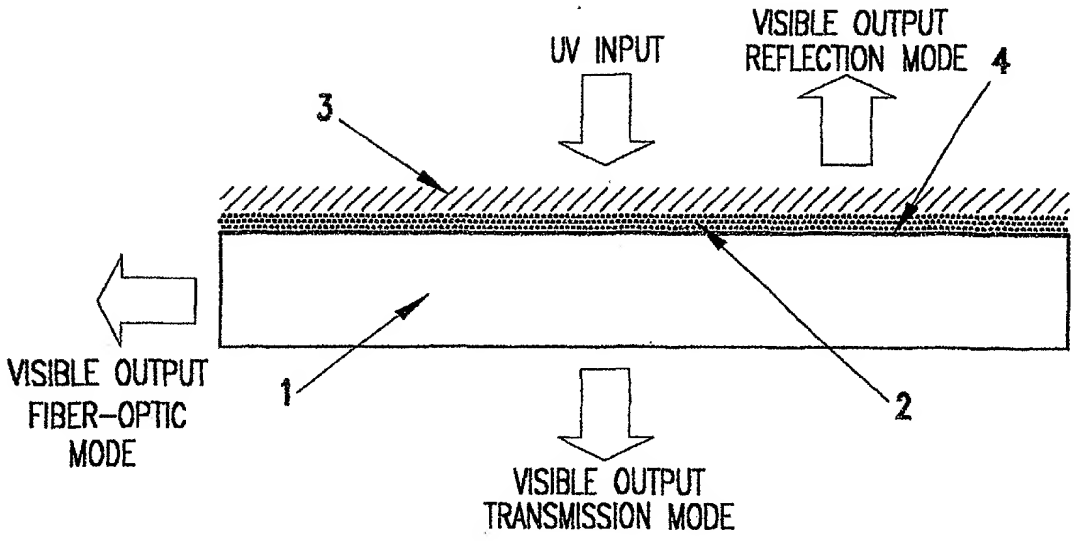


FIG.2A

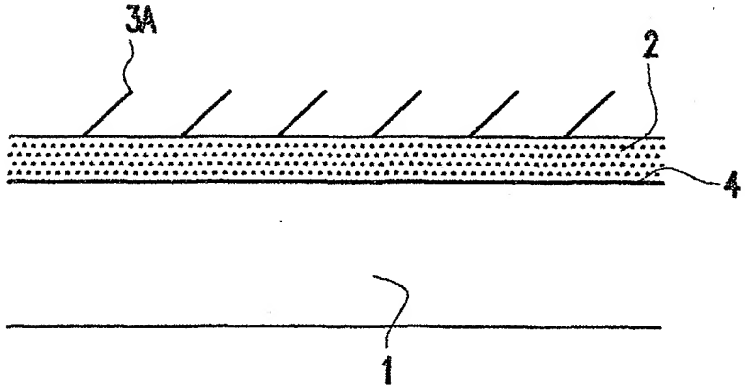


FIG.2B

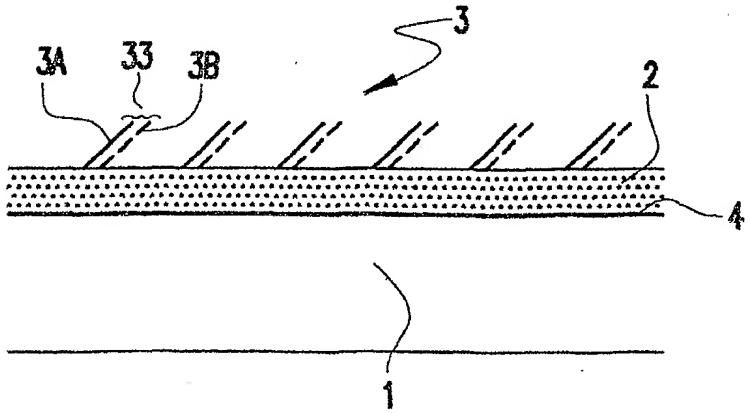


FIG.2C

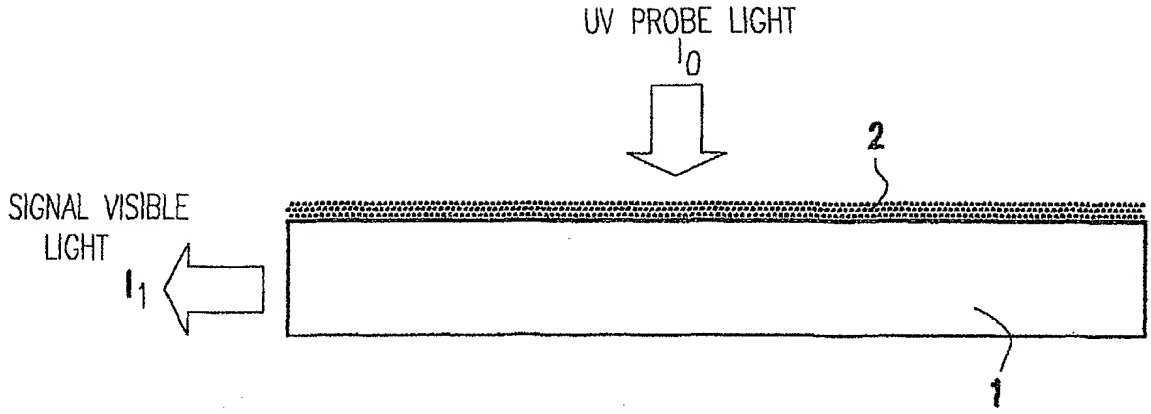


FIG.3A

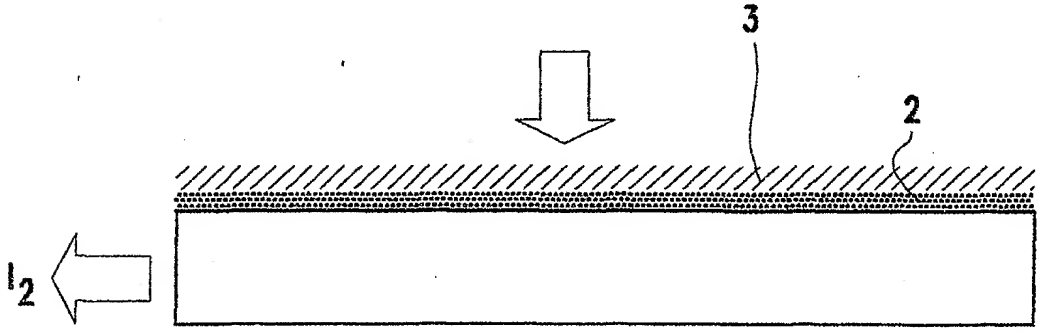


FIG.3B

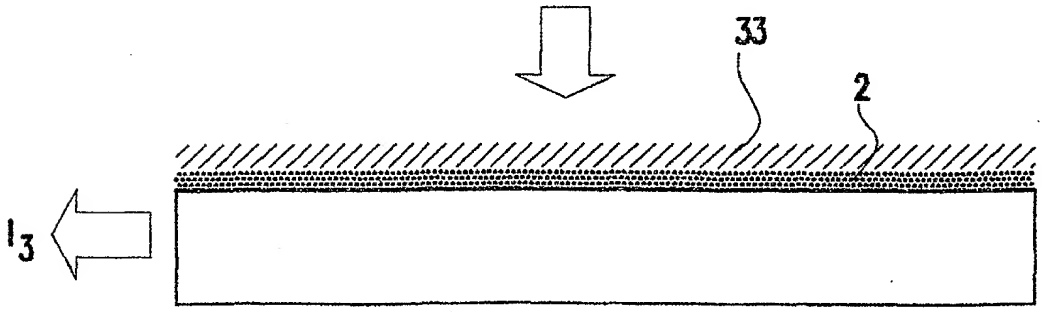
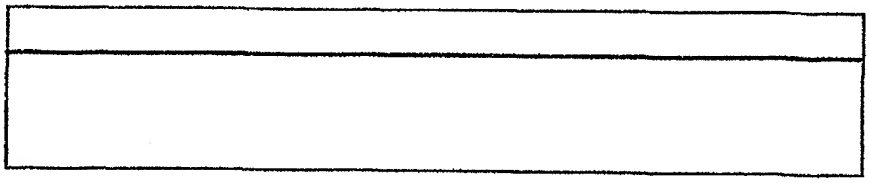


FIG.3C

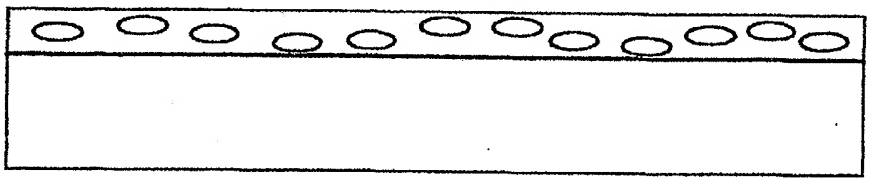
FIG.4A



IN-SITU COMPOSITE

1. ION EXCHANGE
2. PARTICLE SYNTHESIS
3. SURFACE REGENERATION

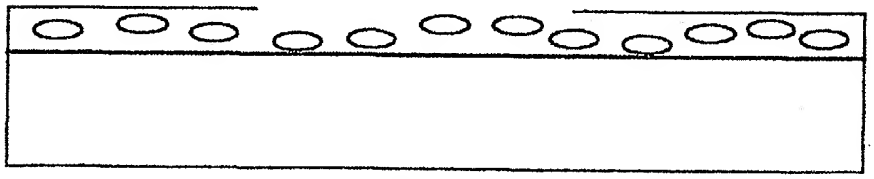
FIG.4B



LITHOGRAPHY

1. SURFACE MODIFICATION AT SELECTED REGIONS

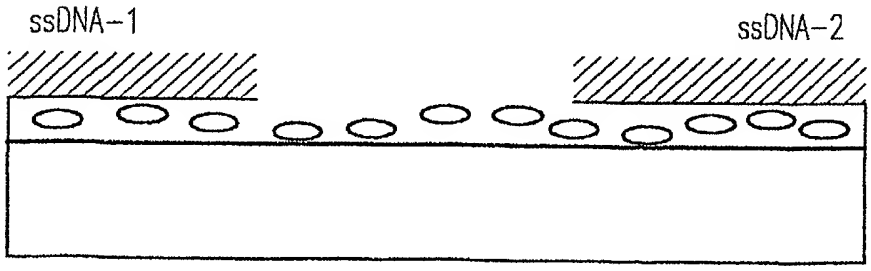
FIG.4C



DNA GRAFTING

1. SELECTIVE ssDNA GRAFTING

FIG.4D



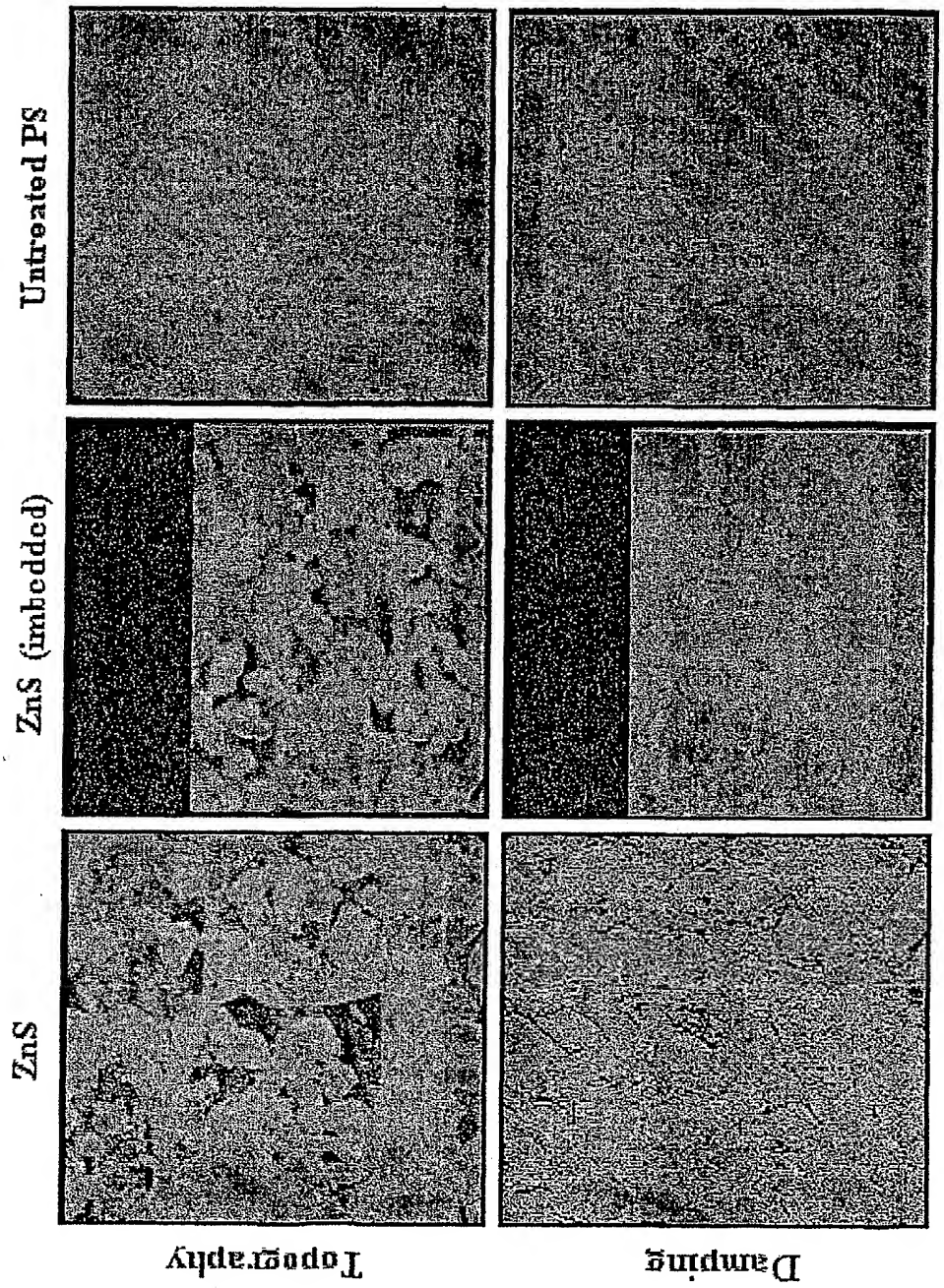
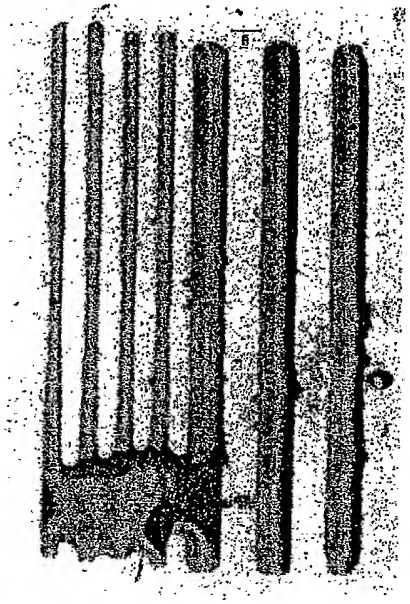


FIG.5



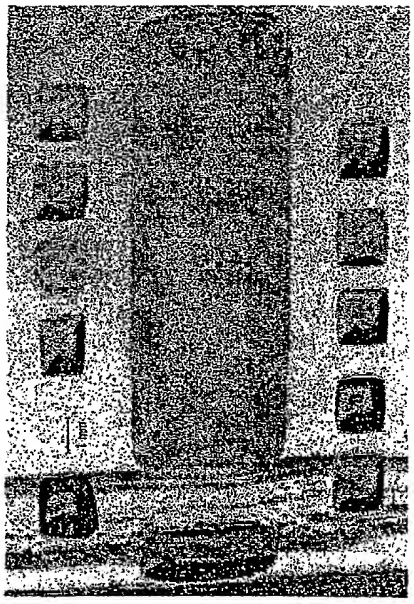


FIG.6

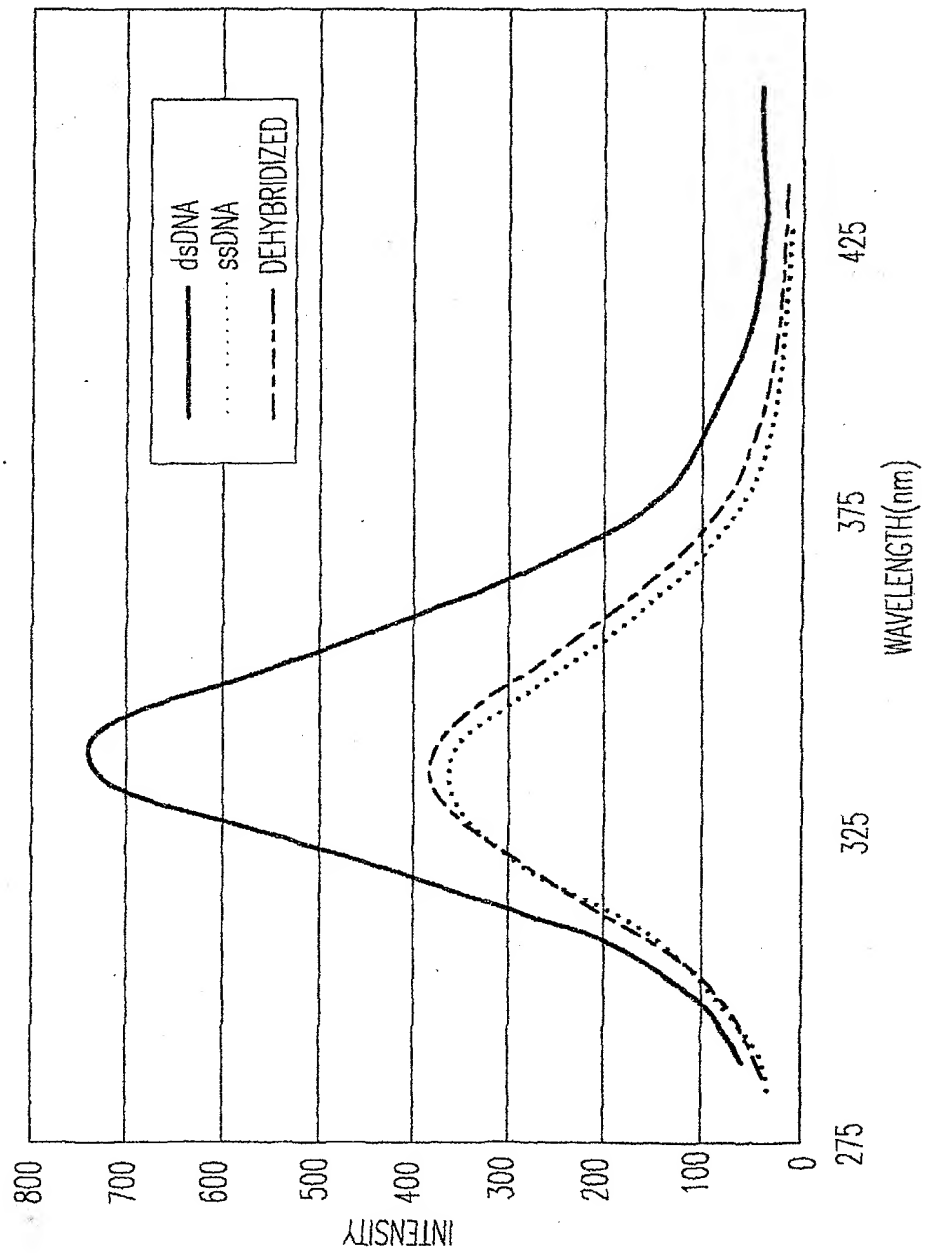

dsDNA
ssDNA
DEHYBRIDIZED
INTENSITY
800
700
600
500
400
300
200
100
0
275
325
375
425
WAVELENGTH(nm)

FIG.7

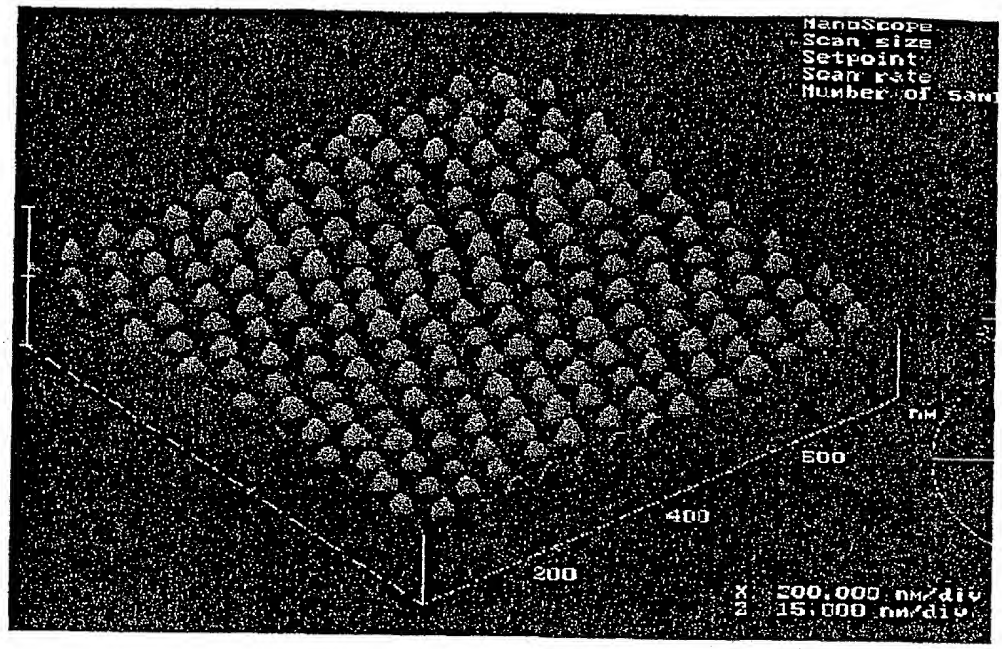

NanoScope
Scan size
Setpoint
Scan rate
Number of Sam
nm
500
400
200
X 200.000 nm/div
Z 15.000 nm/div

FIG.8

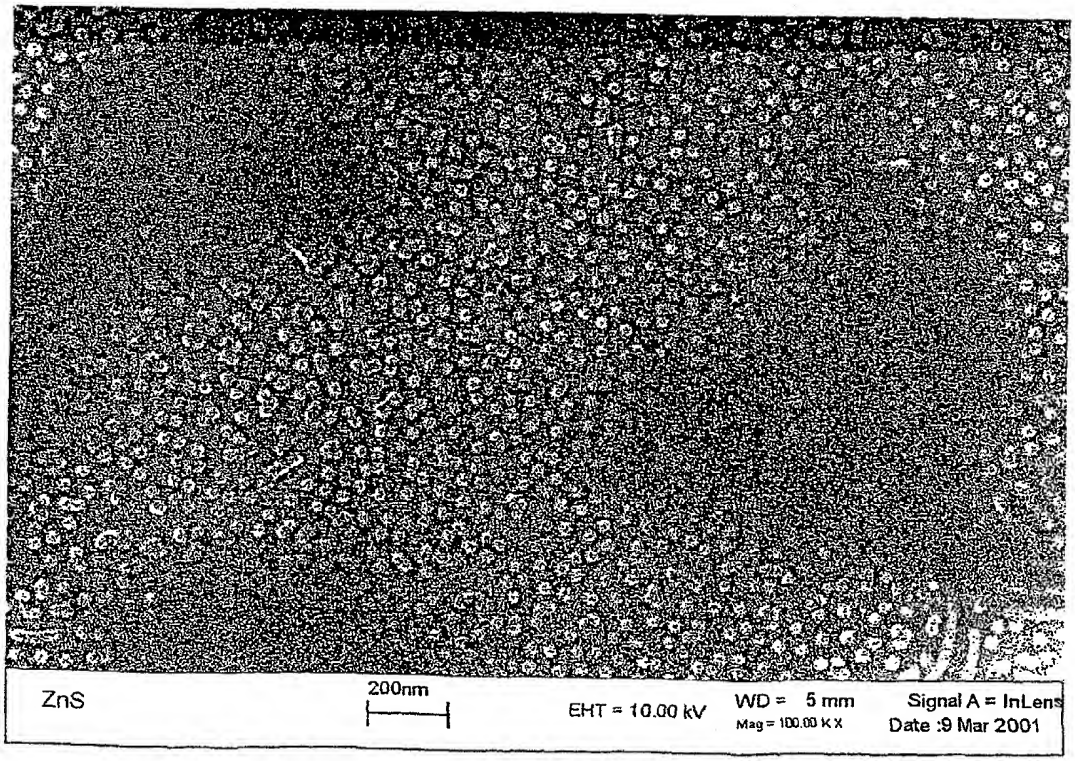

ZnS
200nm
EHT = 10.00 kV
WD = 5 mm
Mag = 100.00 K X
Signal A = InLens
Date :9 Mar 2001

FIG.11

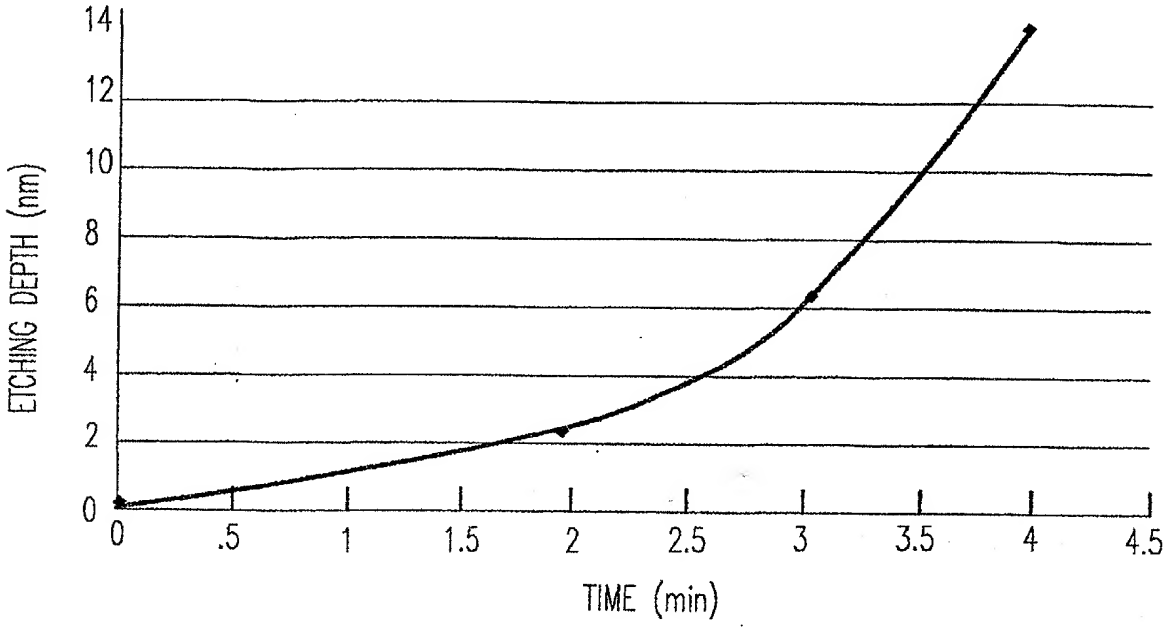

ETCHING DEPTH (nm)
0
2
4
6
8
10
12
14
0
.5
1
1.5
2
2.5
3
3.5
4
4.5
TIME (min)

FIG.9

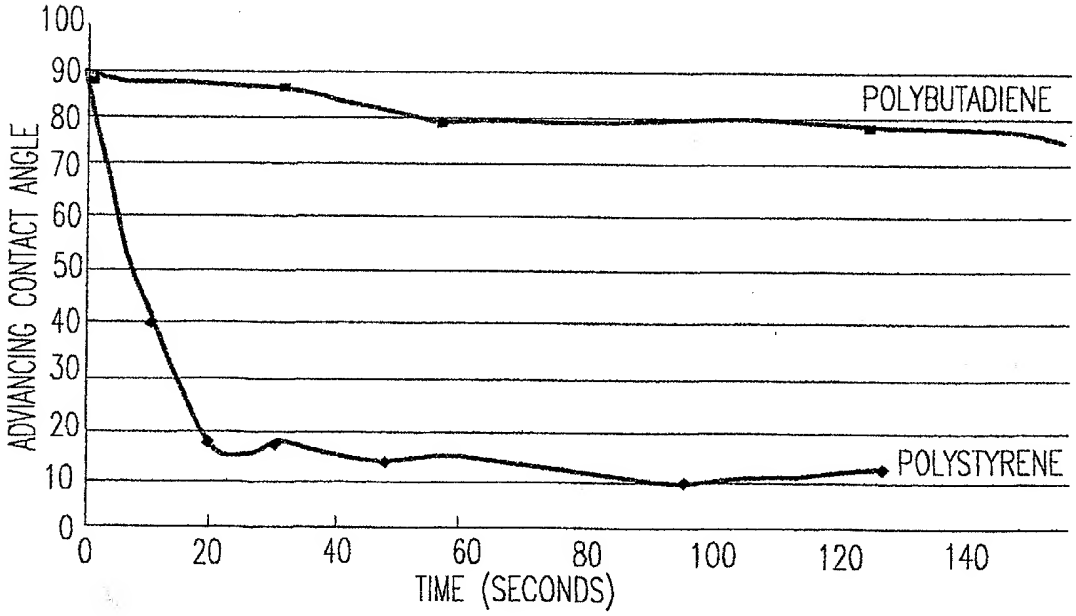

ADVIANCING CONTACT ANGLE
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120
140
TIME (SECONDS)
POLYBUTADIENE
POLYSTYRENE

FIG.10

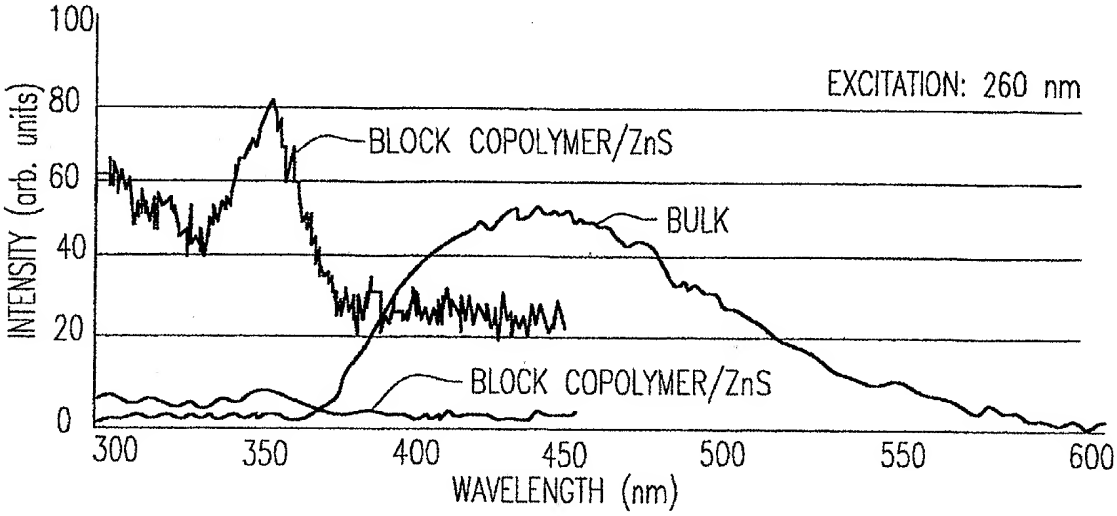

100
80
60
40
20
0
INTENSITY (arb. units)
EXCITATION: 260 nm
BLOCK COPOLYMER/ZnS
BULK
BLOCK COPOLYMER/ZnS
300
350
400
450
500
550
600
WAVELENGTH (nm)

FIG.12

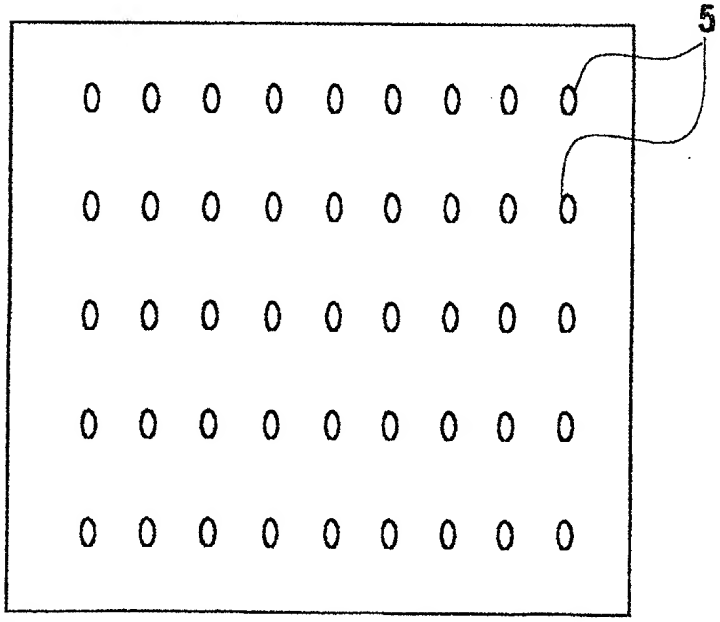

5

FIG.13

FIG.14A

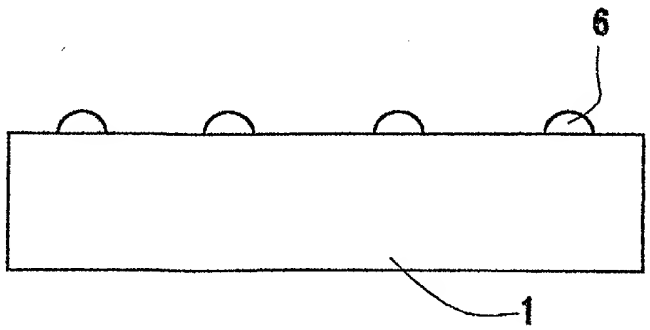


FIG.14B

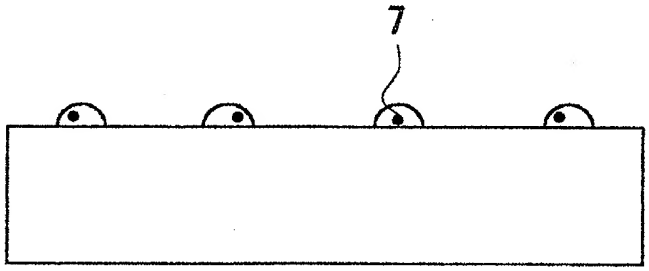
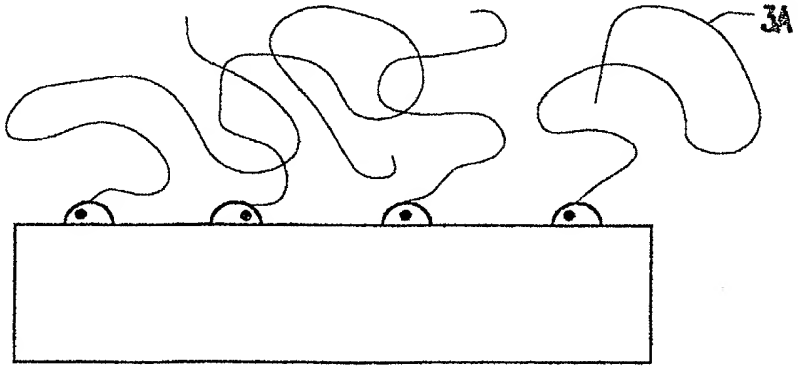


FIG.14C



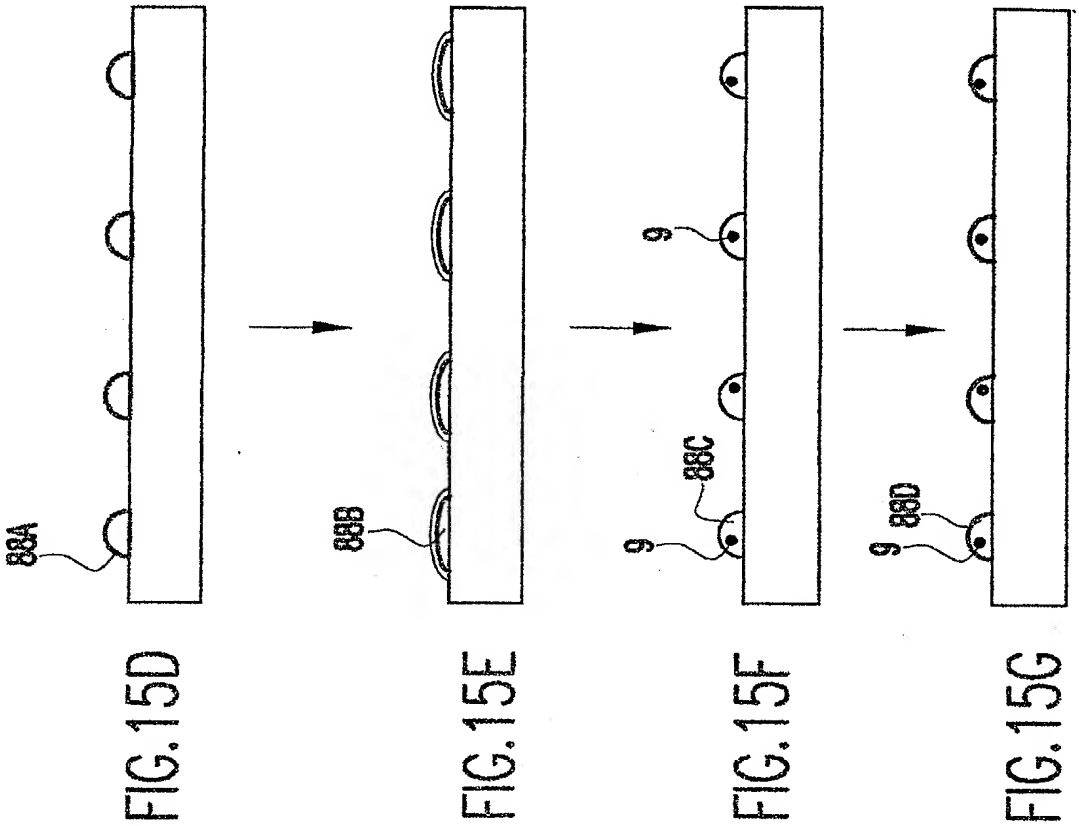


FIG.15D FIG.15E FIG.15F FIG.15G

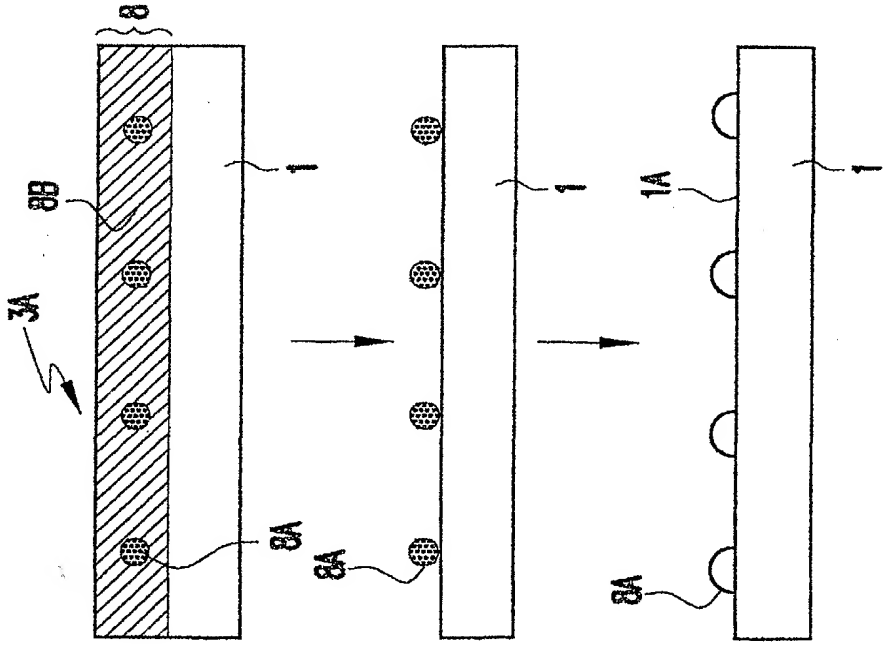
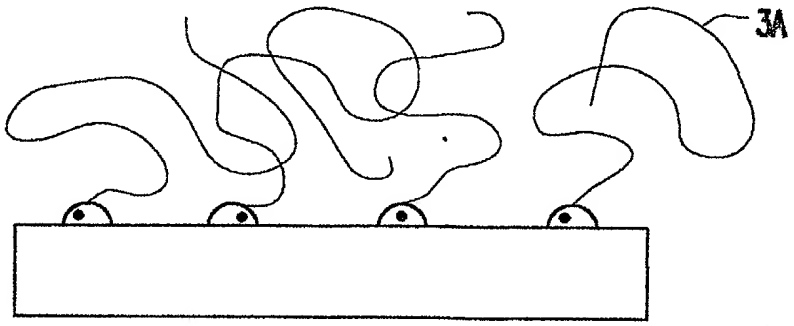


FIG.15A FIG.15B FIG.15C

FIG.16A



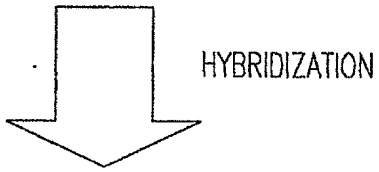
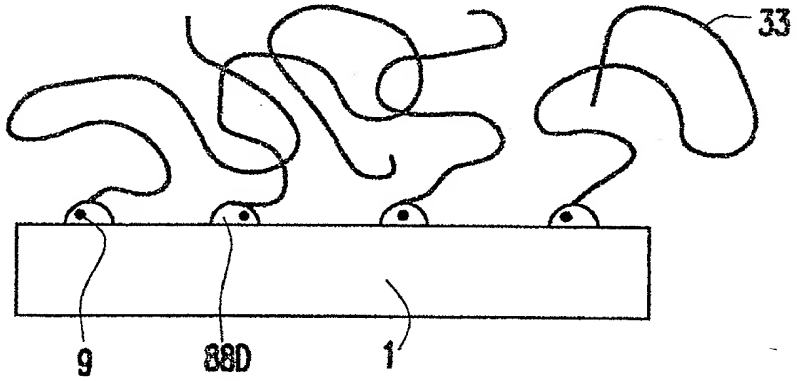


FIG.16B



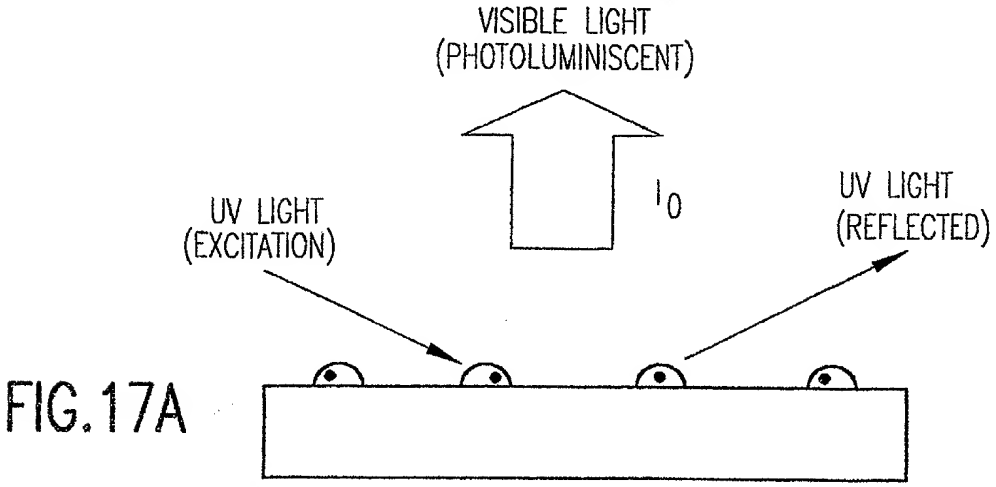


FIG.17A

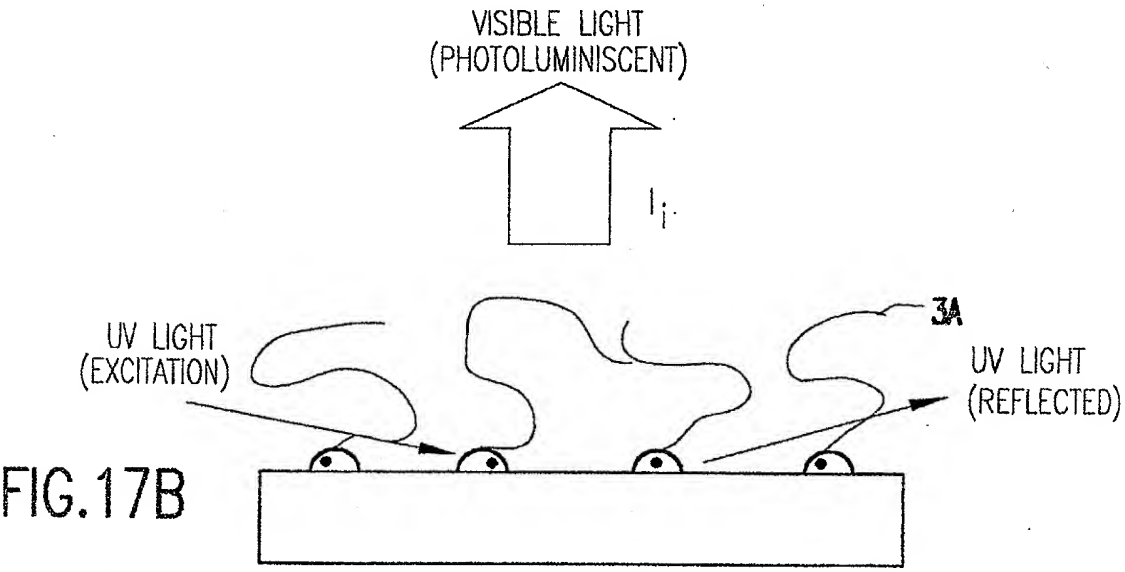


FIG.17B

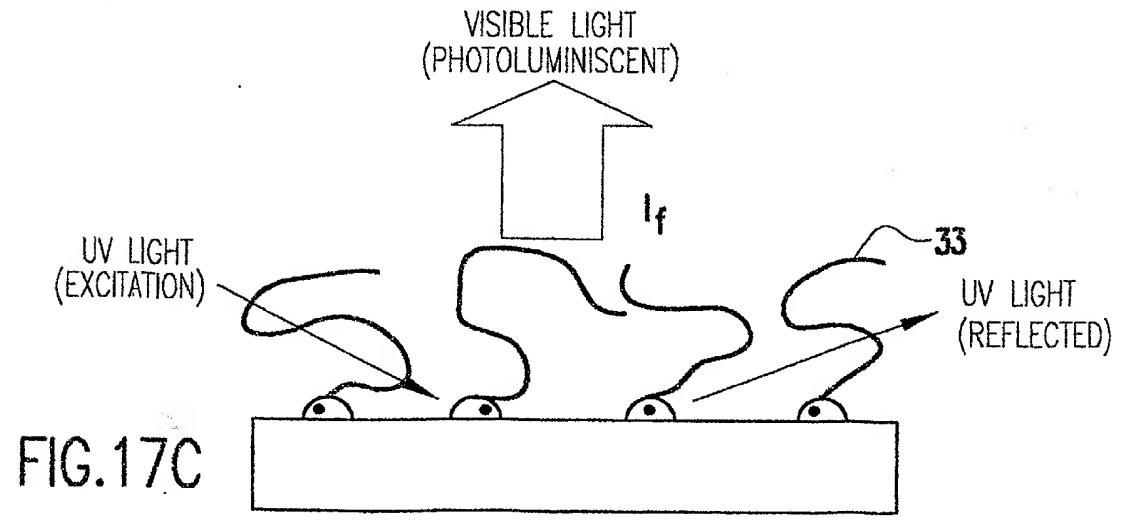


FIG.17C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/30877

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C12Q 1/68
US CL :435/6, 395; 536/22.1, 25.32
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 395; 536/22.1, 25.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, STN, MEDLINE, BIOSIS, CAPLUS, EMBASE
search terms: nucleic, sensor, photoluminescence, tagging

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,981,185 A (MATSON et al) 09 November 1999, see entire document.	1-45
X	US 5,858,653 A (DURAN et al) 12 January 1999, see entire document.	1-45
X	OSTROFF et al. Fixed Polarizer Ellipsometry for Simple and Sensitive Detection of Thin Films Generated by Specific Molecular Interactions: Applications in Immunoassays and DNA Sequence Detection. Clinical Chemistry. June 1998, Vol. 44, No. 9, pages 2031-2035, see entire document.	1-50
X,E	US 6,297,018 B1 (FRENCH et al) 02 October 2001, see entire document.	1-50

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 NOVEMBER 2001	Date of mailing of the international search report 02 JAN 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ARUN CHAKRABARTI Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★